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Increase in Neutralizing Antibody Titer Against Sequential Autologous HIV-1 Isolates After 16 Weeks Saquinavir (Invirase) Treatment

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The humoral immune response to HIV infection plays an important role in determining disease progression. Few and discordant results correlate changes in neutralizing antibody (NtAb) titer with antiretroviral treatment. The NtAb titer against autologous-HIV was evaluated in 33 patients treated with the protease inhibitor saquinavir (SQV, Invirase) and zidovudine (ZDV) alone or in combination. Ten out of 33 (30%) patients showed a significant increase (4-fold or greater) in NtAb titer from baseline in response to the initiation of therapy. A significant correlation ($P = 0.007$) was found between an increase in NtAb titer and treatment with SQV alone (5 subjects) or in combination (5 subjects). A significant decrease in NtAb titer was detected in 7 patients, 5 of whom were treated with ZDV alone. After one year of therapy a significant decrease in HIV-RNA copy number (>0.5 log) with respect to baseline value was detected only in patients treated with SQV alone or in combination. Patients with increased NtAb titer showed a significantly reduced HIV-RNA copy number and increased $CD4^+$ cell count at week 16 of treatment which were sustained up to week 52. These data suggest that treatment with SQV can improve neutralizing activity against autologous virus as well as bring about a significant and sustained reduction in viral load. ***J. Med. Virol. 53:313–318, 1997.***

KEY WORDS: humoral immunity; antiretroviral therapy; neutralization; disease progression

INTRODUCTION

Neutralizing antibodies (NtAb) are a major component of the host defense against viruses, and appear to be particularly important in limiting the spread of cell-free-virus. Results from vaccine trials in animal models suggest that these antibodies may contribute to protection against human immunodeficiency virus (HIV) infection [Poignard et al., 1996]. During the infection, a NtAb response may be detected simultaneously with seroconversion; the titer is low initially [Connick et al., 1996] but increases with time [Albert et al., 1990].

The vigorous neutralizing response in long-term non-progressors is evidence that these patients are exposed constantly to viral antigens, particularly the envelope glycoproteins [Nixon et al., 1992]. The low levels of HIV-1 seen in the peripheral blood samples and lymph nodes of many of these of long-term non-progressors indicate that constant exposure to low-level virus is sufficient to drive a strong antibody response in immunocompetent individuals [Pantaleo et al., 1995; Schonning et al., 1995]. Although NtAb are associated with the healthy carrier state, the infection does persist, and most if not all HIV-1 infected individuals develop AIDS [Arendrup et al., 1992]. This apparent failure of the immune system to control the HIV-1 infection effectively may be due in part to changes in the HIV-1 population driven by NtAb immuno-selection [Albert et al., 1990; Arendrup et al., 1992]. Escape variants of the virus emerge costantly and the NtAb response against autologous viral isolates is highly type-specific [Arendrup et al., 1993]. Thus, in the majority of HIV-positive patients, NtAb against contemporaneous, autologous isolates (recovered from an individual at the same time serum is collected) are generally absent, or present at low titer [Montefiori et al., 1991; von Gegerfelt et al., 1991]. However, sera from HIV-1 infected patients in most cases do not possess detectable neutralizing activity towards late escape virus although they can neutralize earlier virus isolates [Arendrup et al., 1992].

The presence of NtAb against different laboratory strains have been associated with a benign clinical

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course [Robert-Guroff et al., 1988; Robert-Guroff et al., 1993], and patients with lower NtAb titers against a HIV-1_{MN} laboratory strain encountered major clinical events more frequently during follow-up than did patients with higher NtAb titers [Robert-Guroff et al., 1988]. Furthermore, NtAb have also been proposed to reduce the risk of mother-to-child transmission of HIV [Scarlatti et al., 1993]. These data demonstrate that the humoral immune response to HIV infection plays an important role in determining disease progression.

Few and discordant results correlate changes in NtAb titer with antiretroviral treatment. During zidovudine (ZDV) treatment a rapid decline in NtAb titer has been observed [Wainberg et al., 1996] although other reports showed a slight and transient increase [Robert-Guroff et al., 1988; Schimidtmayerova et al., 1992]. Didanosine treatment did not result in any consistent changes in NtAb activity [Robert-Guroff et al., 1988] and titers of NtAb remained stable in lamivudine-treated individuals [Wainberg et al., 1996].

We evaluated the changes of NtAb against autologous virus, viral load and CD4+ cell count in 33 patients enrolled in a double-blinded placebo controlled study which was designed to assess the antiviral activity and tolerability of a HIV proteinase inhibitor, saquinavir (SQV), alone or in combination with ZDV.

METHODS

Study Design

Thirty-three patients with symptomatic HIV infection and CD4+ lymphocyte count ≤300 cells/mm^3, who had not received prior antiretroviral treatment, were enrolled in an evaluation of SQV efficacy. This was a 16-week, parallel, randomized double blind study with blinded monthly extensions of therapy in the absence of major disease progression and toxicity [Vella et al., 1996]. The patients were treated thrice daily with ZDV 200 mg (11 patients), SQV 600 mg (11 patients) and ZDV 200 mg plus SQV 600 mg (11 patients). All patients gave written informed consent and the study received official institutional and ethical approval.

Laboratory Monitoring

Heparinized and EDTA-treated blood samples for plasma viremia titration, HIV-RNA quantitative PCR, HIV-1 isolation, NtAb titration and CD4+ cell count were obtained from patients at baseline and after 16 weeks. Samples for CD4+ cell count and HIV-RNA copy number were also collected at week 52.

Plasma viremia titration and the Roche RT PCR assay to quantify HIV-RNA copy numbers in plasma were carried out according to published techniques [Andreoni et al., 1992; Mulder et al., 1994].

HIV was isolated from plasma as described previously [Sarmati et al., 1994]. Briefly, 1 ml of polyethylene glycol (PEG) pretreated and untreated plasma sample was incubated in T25 flasks with 10^7 PHA-stimulated peripheral blood mononuclear cells (PBMCs), obtained from seronegative donors. Cultures were maintained for 40 days in a humidified chamber

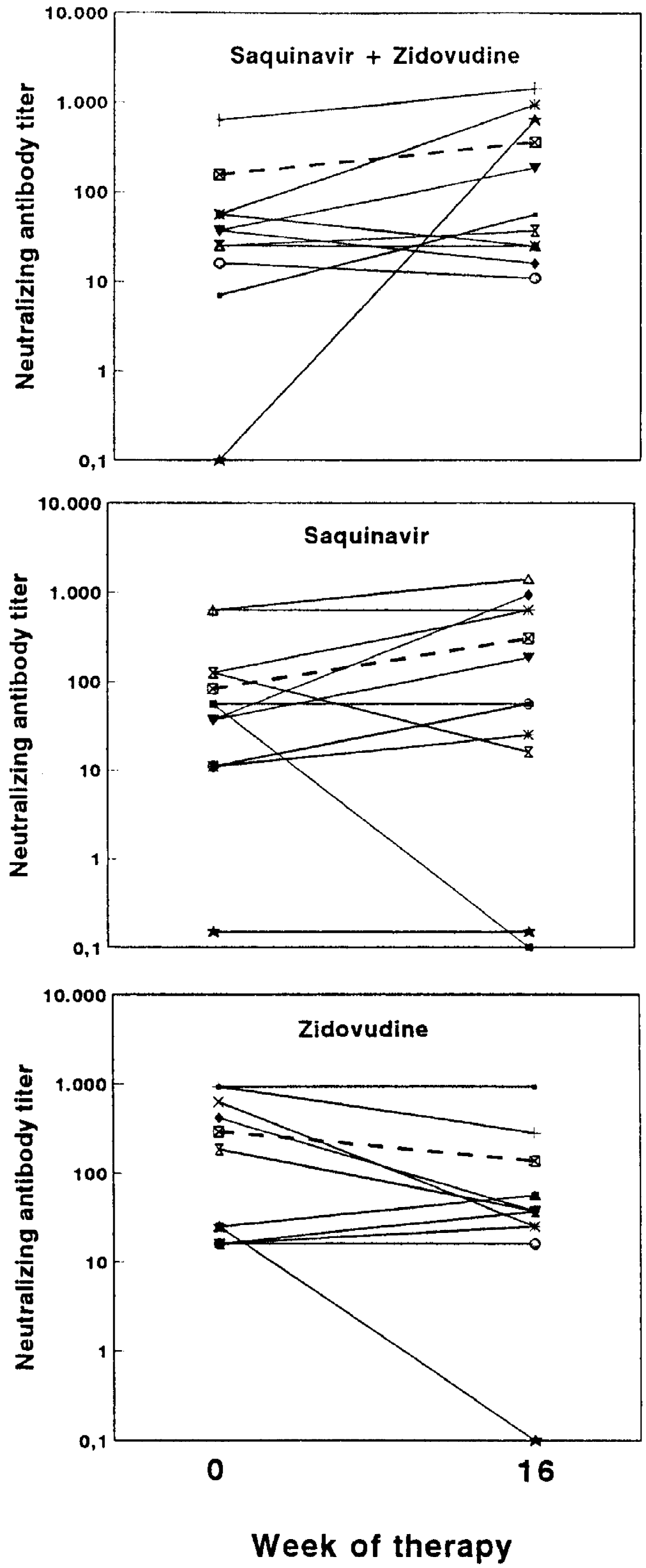

Fig. 1. Neutralizing antibody titer against sequential autologous HIV isolates at baseline and at week 16 of treatment according to the antiretroviral therapy. Sketched lines are representative of the mean titers trends.

TABLE I. HIV-RNA Copy Number and $CD4^+$ Cell Count in Patients Treated with Saquinavir and Zidovudine Alone or in Combination

Treatment (N° patients)	$CD4^+$ cells/ml*			HIV-RNA copy number*		
	baseline	week 16	week 52	baseline	week 16	week 52
ZDV+SQV	141	219	179	173,372	118,664	127,262
(11)	(±85)	(±138)	(±86)	(±226,508)	(±159,568)	(±288,154)
ZDV	180	204	152	179,645	133,734	174,262
(11)	(±107)	(±140)	(±123)	(±239,728)	(±130,795)	(±168,478)
SQV	160	212	214	347,825	149,090	181,626
(11)	(±106)	(±127)	(±150)	(±279,040)	(±161,378)	(±192,423)

*Mean ± SD. ZDV = zidovudine; SQV = saquinavir.

TABLE II. Correlation of HIV-RNA Copy Number and $CD4^+$ Cell Count with NtAb Titer Trends During 52 Weeks of Antiretroviral Treatment

NtAb titer (N° patients)	$CD4^+$ cells/ml*			HIV-RNA copy number*		
	baseline	week 16	week 52	baseline	week 16	week 52
Decreased	146	139	117	314,490	237,043	307,780
(7)	(±126)	(±124)	(±112)	(±311,759)	(±255,127)	(±288,154)
Unchanged	161	209	166	236,078	135,965	174,262
(16)	(±89)	(±130)	(±111)	(±237,452)	(±137,199)	(±168,478)
Increased	170	267	253	232,896	119,024	145,206
(10)	(±100)	(±124)	(±112)	(±210,256)	(±139,475)	(±140,638)

*Mean ± SD. NtAb = neutralizing antibody.

at 37°C, with 5% CO_2 and monitored twice weekly for p24 antigen production using a commercially available enzyme immunoassay (ELISA, Abbott Laboratories, North Chicago, IL, USA). A culture was considered positive if the concentrations of p24 exceeded 1000 pg/ml in two consecutive determinations. Positive supernatants were harvested by centrifugation and stored in liquid nitrogen.

To determine whether the HIV isolates were syncytium-inducing (SI) or non-syncytium-inducing (NSI), an aliquot of viral stock supernatant, containing 100 $TCID_{50}$/ml, was cultured with 10^6 MT-2 cells. Cultures were maintained for 4 weeks and were examined for syncytia twice weekly [Japour et al., 1994].

Neutralizing Antibodies Titration

NtAb titer was assayed against autologous virus isolated at the same time as serum. All sera were tested against all virus isolates on the same day using the same pool of PHA-stimulated PBMCs as target cells. Sera were inactivated for 30 min. at 56°C, diluted in medium (RPMI) by four steps of five fold dilutions. One hundred ml of each dilution were added in triplicate to 96-well culture plates. A standard viral dilution containing 100 $TCID_{50}$/100 ml, or medium, was then added in an equal volume and the plate was incubated for 1 hr at 37°C. Subsequently 10^5 PHA-stimulated PMBCs in 100 ml were added and the plate was further incubated overnight. One hundred microliters of the medium were changed after 3 days and after 7 days culture supernatants were analyzed for p24 antigen. Sera from two sero-negative subjects were used as negative serum controls. The neutralizing titer was calculated by interpolation (Reed & Muench, 1938) as the reciprocal of the dilution which reduced the number of infected cultures by 50%.

Statistical Analysis

All contingency tables were analyzed by Fisher's exact test. Joint analysis of 2 × 2 tables, stratified by NtAb titration, was performed by the Mantel–Haenszel Chi-square test. All P-values are two-sided. Group means were compared by analysis of variance. If the corresponding F-test was statistically significant then individual means were compared using the Bonferroni additive inequality procedure, which controls for the maximum experimental error rate (α).

RESULTS

NtAb Titer Response During Antiretroviral Therapy

Changes in NtAb titer from baseline to week 16 of therapy according to the antiviral treatment are reported in Figure 1. At baseline no significant difference in NtAb titer was detected among the 3 groups of patients even though subjects treated with ZDV alone had the highest NtAb titer against autologous virus. A significant correlation ($P = 0.007$) was found between NtAb titer trend and antiretroviral regimen. An unequivocal increase in mean NtAb titer was detected in the group of subjects treated with SQV alone (from 83 to 305) and in combination with ZDV (from 156 to 358). A particularly significant increase (4-fold or greater) change from baseline NtAb titer in response to initiation of therapy was observed at week 16 in patients treated with the proteinase inhibitor alone (five subjects), or in combination therapy (five subjects). Conversely, patients treated with ZDV alone showed a dis-

tinct decrease in mean NtAb titer (from 293 to 136). Furthermore, no patient treated with combination therapy showed a significantly decreased (4-fold or greater change) NtAb titer, while 5 out of 11 patients (45%) treated with ZDV alone and 2 out of 11 patients (18%) treated with SQV alone showed a significant decrease in NtAb titer.

Viral Load and CD4+ Cell Count During Antiretroviral Therapy

Changes in HIV-RNA copy number and CD4+ cell count during antiretroviral treatment are reported in Table I. At baseline no significant difference in viral load and CD4+ cell count was detected among the 3 groups of patients treated with ZDV, SQV and ZDV plus SQV. Moreover, before treatment a similar prevalence of subjects with syncytium-inducing HIV isolates was detected among the three groups of patients (36%, 54% and 36% respectively).

In all patients a decrease in viral load and an increase in CD4+ cell count was detected at week 16 of treatment. After one year of therapy a significant reduction in plasma HIV-RNA copy number (>0.5 log) with respect to baseline values was detected only in patients treated with SQV alone (9 subjects) or in combination (7 subjects).

Correlation Between Changes of NtAb Titer, CD4+ Cell Count and Viral Load

Table II shows changes in CD4+ cell count and viral load in relation to NtAb titer. At baseline no significant difference was detected between patients with increased, unchanged and decreased NtAb titer, even if patients with decreased NtAb titer had the highest HIV-RNA copy number and lowest CD4+ cell count. At weeks 16 and 52 of treatment, a significant increase in CD4+ cell count ($P = 0.004$ and $P = 0.005$ respectively) was detected only in patients with an increased NtAb titer. After 16 weeks of treatment, a reduction in HIV-RNA copy number was observed in all groups of patients, but a significant decline was detected only in subjects with an increased or unchanged NtAb titer ($P = 0.04$). However, at week 52 only patients with increased NtAb titer showed a significant decrease in viral load from baseline. Furthermore, after 16 and 52 weeks of treatment patients with increased NtAb titer had a significantly lower HIV-RNA copy number ($P = 0.03$ and $P = 0.01$, respectively) and higher CD4+ cell count ($P = 0.05$ and $P = 0.03$, respectively) respect to patients with decreased NtAb titer.

Figure 2 shows the correlation among changes in NtAb titer at 16 weeks of therapy and variations of CD4+ cell count and viral load at week 52 in the three different treatment groups. Among the 10 patients with a significant increase of NtAb titer, 9 subjects showed a significant reduction in HIV-RNA copy number (>0.5 log) and 7 subjects had an increase of more than 50 CD4+ cells/mm^3. Moreover, among the 7 patients with a significant decline of NtAb titer, only 1 individual (treated with saquinavir alone) showed a

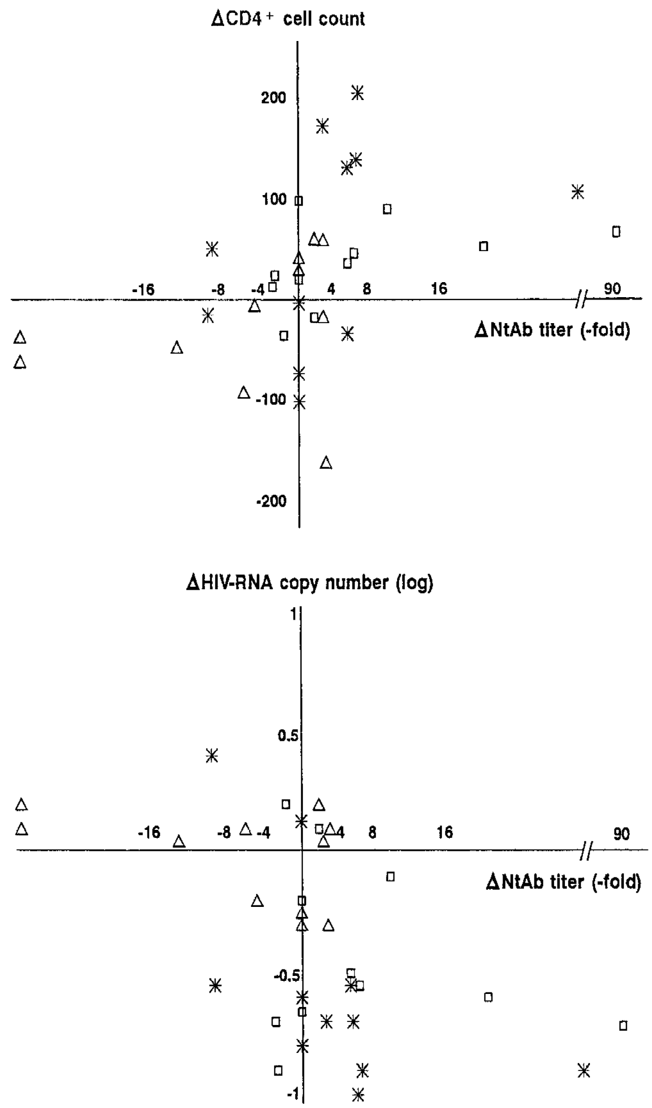

Fig. 2. Correlations between changes of CD4+ cell count and viral load after 52 weeks of therapy according to variations of NtAb titer at 16 weeks of treatment. *Patients treated with SQV alone. △Patients treated with ZDV alone. □Patients in combination therapy.

significant reduction in HIV-RNA copy number and an increase of more than 50 CD4+ cells/mm^3.

DISCUSSION

The effects of ZDV and SQV alone or in combination were examined on the capacity of sera to neutralize sequential autologous HIV isolates in 33 HIV-seropositive treatment-naive subjects.

A significant correlation was found between an increase in NtAb titer and protease inhibitor treatment. In particular, 45% of patients treated with SQV alone or in combination showed a significant increase in NtAb titer; conversely, like other investigators [Wainberg et al., 1996] a rapid decline in NtAb titer was found in 45% of patients treated with ZDV alone. No data are available on the potential benefit of restoring NtAb activity in advanced infection. In our naive pa-

tients with less than 300 $CD4^+$ cells/mm^3, the application of therapy coincided with an improvement in parameters.

At week 52 of treatment, a significant decrease in viral load and increase in $CD4^+$ cell count was detected only in patients with increased NtAb titer. These data suggest that neutralizing activity may provide some control over the progression of HIV infection. Indeed, high NtAb titers are usually associated with the asymptomatic phase of HIV infection, and a decline in neutralizing activity is linked with the impairment of the humoral immunity system and clinical progression to AIDS [Arendrup et al., 1992; von Gegerfelt et al., 1991; Montefiori et al., 1996]. Furthermore, HIV-1 infected LTNPs have shown higher NtAb titers to HIV laboratory strains and to primary isolates compared with progressors [Montefiori et al., 1996].

Sera from the majority of patients with advanced disease could still neutralize the initial primary isolates effectively but could not neutralize subsequent autologous isolates [Tsang et al., 1994]. This suggests that new virus variants continue to stimulate the immune system to produce antibodies to the initial primary HIV isolates but not to themselves. Therefore, as demonstrated in vitro [Reitz et al., 1988], HIV variants that evolve in vivo may evade the immune system as a selected population of related genomes unrecognized by NtAb.

The reduction in viral burden observed during antiretroviral therapy could drive a slower rate of evolution of HIV-1 quasispecies followed by the improvement of neutralizing activity against autologous isolates. In the present, an increased NtAb titer was detected in patients with the highest reduction in HIV-RNA copy number. Moreover, the high proportion of defective virus particles expected during protease inhibitor treatment could have immunomodulatory effects and drive a stronger NtAb response.

In future, it will be important to detect the changes in the envelope sequences that are involved in escape from neutralization. Additional work is needed to determine which quasispecies are present at the initiation of therapy and which of these become selectively amplified during treatment. It will also be critical to compare the dominant isolates in vitro with the species that are present in vivo [Tsang et al., 1994].

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